

TITLE

Rapid throughput concentration-response analysis of human taste discrimination

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Nonstandard abbreviations: ACE K, acesulfame potassium; LsA, Limits of Agreement; MCS, Method of Constant Stimuli; MSR, Minimum Significant Ratio; REB A, rebaudioside A; SDT, Signal Detection Theory; TAS1R, Taste 1 Receptor; TAS1R2/R3, Taste 1 Receptor 2+Taste 1 Receptor 3 heterodimer; TAS2R, Taste 2 Receptor; TSD, touch-sensitive display.

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ABSTRACT

Human taste threshold measurements often are used to infer tastant receptor functionality. However, taste thresholds can be influenced by receptor-independent variables. Examination of the full range of taste-active concentrations by taste discrimination has been hampered by logistics of testing multiple concentrations in replicate with human subjects. We developed an automated rapid throughput operant methodology for taste discrimination and applied it to concentration-response analysis of human taste. Tastant solutions (200 μ l) drawn from a 96-well plate and self-administered to the tongue served as discriminative stimuli for money-reinforced responses on a touch-sensitive display. Robust concentration-response functions for "basic taste" stimuli were established, with particular focus on agonists of the TAS1R2/R3 receptor. With a training cue of 100 mM sucrose, EC₅₀s of 56 μ M, 79 μ M, 310 μ M, and 40 mM were obtained for rebaudioside A, sucralose, acesulfame potassium, and sucrose, respectively. Changing the sucrose training cue to 300 mM had no impact but changing to 30 mM resulted in slight leftward shifts in potencies. A signal detection method also was used to determine values of d' , a probabilistic value for discriminability, which indicated that 5 mM was near the limits of detection for sucrose. With repeated testing, both EC₅₀s and 5 mM sucrose d' values were established for each individual subject. The results showed little correspondence between threshold sensitivities and EC₅₀ values for sucrose. We conclude that concentration-response analysis of taste discrimination provides a more reliable means of inferring receptor function than measurement of discriminability at the lowest detectable tastant concentrations.

SIGNIFICANCE STATEMENT

Many inferences about human tastant receptor functionality have been made from taste threshold measurements, which can be influenced by variables unrelated to receptors. We herein report a new methodology that enables rigorous concentration-response analysis of human taste discrimination, and its use toward quantitative characterization of tastant agonist

activity. Our data suggest that taste discrimination concentration-response functions are a more reliable reflection of underlying receptor activity than threshold measures obtained at the lowest detectable tastant concentrations.

INTRODUCTION

Taste quality refers to the sensory recognition of a chemical stimulus applied to the tongue. Using traditional terminology, there are five “basic” taste qualities—sweet, bitter, umami, salty and sour. Taste qualities are inferred from the behavioral capacity for discriminating among tastants (Palmer, 2019). On the physiological level, taste discrimination is enabled by distinct subpopulations of specialized sensory cells within the taste bud that each are committed to the faithful propagation of signals translating into individual taste qualities (Yarmolinsky et al., 2009). Receptors selective for different tastant molecules are differentially expressed in each subpopulation; for example, TAS2R receptors are almost exclusively expressed on bitter-sensing cells (Voigt et al., 2012), and similarly, TAS1R2/R3 receptors on sweet-sensing cells (Nelson et al., 2001). Thus, it is the cell that determines the taste quality, but the receptor that determines which tastant agonists can stimulate a taste cell and the concentration ranges of agonist activity (Zhao et al., 2003). Therefore, *in vivo* assays of taste discrimination can be used to study the pharmacology of tastant receptors, particularly since there is relatively little in the way of pharmacokinetics that would obscure interpretations of the results.

Objective measurement of taste discrimination is accomplished through methods in which subjects choose between two or more samples to indicate detection of the taste property of interest. Many trials must be executed to quantify the discrimination with statistical reliability. Highly efficient animal models of taste discrimination have been developed using operant procedures, where up to hundreds of trials can be conducted within single test sessions of an hour or less (Perez et al., 2013). In operant taste discrimination, an antecedent tastant stimulus indicates which of two or more behavioral tasks will be reinforced, as for example, when reinforcement of a rat’s lever presses occurs on one lever after tasting water and on an alternative lever after tasting sucrose (Morrison, 1969; Palmer et al., 2013). Once the taste discrimination is established, the distribution of lever presses occurring on trials of novel stimuli

then can be taken as a quantification of commonality with the taste properties of a standard. Concentration-response relationships are readily generated by these models, amenable to pharmacological analysis (Palmer et al., 2013).

Methods for measuring taste discrimination in humans are less efficient, with few samples evaluated per session. Test samples typically are presented as solutions in cups, ranging in volumes from 5 to 30 ml (for example (McBride, 1983; Mennella et al., 2014), or as tastant-soaked swabs applied to specified locations on the tongue surface (for example (Heath et al., 2006; McMahon et al., 2001). The logistics of preparing test samples and manually presenting them to subjects become self-limiting with increasing numbers of trials. Additional rationale for limiting trial numbers in human taste discrimination tests stems from a prevailing concern over a decline in performance due to “boredom” and/or fatigue (Linschoten et al., 2001; Meilgaard, 2016). The practicalities of human taste measurement therefore tend to dissuade against studying discrimination across the entire taste-active range of concentrations.

Regardless of logistics, the number of tastant concentrations examined in an experiment often is restricted by design, to just a few near the limits of sensory detection for the purpose of establishing a sensory threshold (Lawless, 2013). Many published studies report the use of taste discriminate procedures to obtain threshold values, taken as an indication of the “sensitivity” of the taste system under investigation. But relatively few studies of human taste discrimination have tested a concentration range that reflects the expected span of receptor occupancy. It is not clear how tastant sensitivities as they have been reported relate to the rest of the concentration-response function; specifically, whether differences in responsiveness at low, threshold concentrations of tastant agonist distribute to all taste-active concentrations. Without examining the entire range of taste-active concentrations, the relationship between the human sensory response and the underlying receptor pharmacodynamics remains elusive.

We herein report the development of an automated technology and assay for rapid throughput taste discrimination designed for human subjects, capable of generating concentration-response functions from the full taste-active concentration range of a tastant agonist. We have applied this technology to obtain robust concentration-response functions for human taste discrimination of multiple tastant agonists, with particular focus on TAS1R2/R3 agonists, within single test sessions. We further used the approach to address some open questions about the relationship between taste stimulus thresholds and the rest of the taste discrimination concentration-response function for sweet tasting agonists, especially sucrose.

METHODS

General Methodology

Apparatus and software were constructed and developed according to our design by Biomated Solutions (Randolph, NJ). The system is comprised of an automated sample delivery device and a laptop computer with touch-sensitive display (TSD; Figure 1A, 1B, Supplemental Videos S1 and S2) that communicates by WiFi with a cloud-based server. The server hosts an interactive software application that runs the training and test sessions and records subjects' responses, and a database that stores generated data. The entire system is known commercially as TāStation®.

The methodology of the TāStation system follows the paradigm of operant taste discrimination. Subjects are trained to perform an operant task after tasting one or multiple solutions that serve as discriminative cues. The operant task is touching a target within a demarcated region (the response field) on the TSD that has been associated through training with the taste of a specific stimulus solution. The target is defined by a central pair of x,y coordinates surrounded by three concentric circles (emulating a dart board). A consequence occasions the subject's response; either a positive reinforcer for correctly matching the taste stimulus with its designated

coordinates in the response field of the TSD, or a penalty for touching outside the outermost radius (explained in detail below).

The positive reinforcer is a virtual poker chip appearing on the TSD that represents an actual monetary value. The magnitude of monetary reward depends on where in the target the subject touches. The greatest value is returned for touches in the center of the target (the bullseye). The value of the reward decreases progressively as touches occur in the outer two rings of the target. Touches made altogether outside of the target result in a penalty—an immediate subtraction of money earned thus far in the game. The number of targets, their size and location, and monetary values defining positive reinforcers and penalties all are variables that are entered into the program for a specific experimental design. The targets are not displayed on the response field and therefore subjects must discover their location through trial-and-error during the training sessions.

For all trials during a training session, the subject must correctly match the taste stimulus with its designated target in order to receive the monetary reward and avoid the penalty. Once the discrimination performance has reached an accuracy of at least 90% correct responses, subjects then are advanced to a test session. The purpose of a test session is to quantify the similarity or disparity of the taste qualities of novel stimulus solutions as compared to the discriminative training cues. Therefore, the training cues are included in the test sessions along with the novel stimulus solutions. To retain stimulus control, positive reinforcement (and avoidance of penalty) remains contingent on touching the stimulus-appropriate target on trials of training cue solutions (here forward defined as *control standard trials*). On trials of novel taste stimuli (i.e., any taste stimulus that is not a control standard, here forward referred to as *test articles*) a maximal value positive reinforcer occasions all touches placed anywhere within the response field of the TSD.

Apparatus

The sample delivery device is a single unit that rests on a table (Figure 1A, 1B, Supplemental Video S1); the footprint of the unit is 28 cm wide by 35.5 cm deep and has an overall height of 48.5 cm, and overall weight of 11.4 kg. A belt-driven x-y motion table is housed in a cabinet 14 cm in height by 26 cm in length in the base of the unit.

A plastic tray designed to securely hold a standard 96-well plate is centered on and fixed to the motion table. On the front end of the tray, just outside the depression for the 96-well plate, there is a hole fit to the outer dimensions of a 1.5 ml Eppendorf tube; during training and test sessions the tube is filled with water for washing the pipette tip between trials. When seated in the tray of the x-y motion table, the top of the 96-well plate is 3 mm below the bottom surface of the cabinet housing, and the cabinet housing is 8 mm thick.

A vertical extension of the base, measuring to a height of 34.5 cm above cabinet, supports a z-axis gantry. A 3-D printed clasp is attached to the z-axis gantry. The clasp is designed to hold an electronic, programmable Viaflo single channel pipette (Integra, Hudson, NH) with Bluetooth communication capacity. When clipped into the clasp, the pipette is positioned over the cabinet in the unit base where the x-y motion table is housed. A removable lid serves as a cover for the cabinet when the system is in operation. A hole (1 cm dia.) is aligned beneath the pipette in the clasp so that the pipette's tip (300 ul volume capacity, sterile, filtered Integra Grip Tip, Integra, Hudson, NH) can be automatically lowered through the hole and into a single well of a 96-well plate nested in the x-y motion table. When the z-axis gantry is in the initial ready position, the pipette tip is 3 cm above the surface of the x-y motion table cabinet lid.

The laptop communicates operations to the x-y motion table and z-axis gantry by an ethernet cable, and to the electronic pipette by Bluetooth. The laptop communicates continuously via WiFi to a cloud-based server that hosts a set of interactive algorithms designed to carry out all

operations of taste testing and training from session start to finish—operating the sample delivery system, registering and storing subject responses, trial-by-trial, in real time.

The response field appearing on the TSD is a visually demarcated rectangular area (14.5 cm height x 15.5 cm width) covering the left half of the display (Figure 2). Underlying the response field is a virtual Cartesian field in which the coordinates are enumerated as proportions of distance along the abscissa and ordinate. The ordinate in this scheme is reversed from that customary to mathematics, so that values increase from top to bottom along the number line. Thus, the central coordinate pairs for quadrants I, II, III and IV are (0.75, 0.25), (0.25, 0.25), (0.25, 0.75), and (0.75, 0.75), respectively, and the origin is at (0.5, 0.5). For the experiments described herein, the abscissa and ordinate were projected within the response field, each also with hatch marks (the hatch marks did not correspond to any numeric values but were included as visual reference to aid target location; Figure 2). An 8 x 12 matrix, representing a 96-well plate, is projected to the right of the response field, the purpose of which is to provide subjects a record of their scores as they advance trial-by-trial through the game-like sessions. The consequence of the response on any given trial is indicated by a blue poker chip (positive consequence) or a red “X” (negative consequence) appearing in the well immediately upon the trial’s completion. Just below the matrix are two counters, one on the left side indicating the trial number, and one on the right side indicating cumulative points earned (Figure 2, Supplemental Video S2)). In the current study, the value of each point equaled \$0.01.

All software programming for the TāStation system was accomplished using LabView (National Instruments, Austin, Texas) system-design platform and development environment.

Testing Site

Training and testing occurred at Opertech Bio located in the Pennovation Center of the University of Pennsylvania (Philadelphia, PA, USA.) The testing suite was an enclosed 20 square meter room illuminated by fluorescent lighting and ambient light through large windows. The room contained three TāStation systems placed approximately 1.5 m apart, each on the surface of a 60 cm x 120 cm table area. Appointments for training and testing sessions were scheduled individually for each subject, and 1 to 3 subjects occupied the room at any given time when each was engaged in their session. Subjects were seated directly in front of a TāStation system with the sample delivery device to their left and laptop to their right, both components within easy reach.

General Procedure for Current Studies

Materials

Sucrose (Domino Granulated Sugar), was purchased at a local grocery store, quinine HCl, NaCl, and citric acid were purchased from Sigma Aldrich (St. Louis, MO, USA), sucralose was procured from Tate & Lyle (London, UK), acesulfame potassium (ACE K) from Celanese (Dallas, TX) and rebaudioside A 97% (REB A) from Prinova (Carol Stream, IL). All tastants were dissolved in Deer Park Brand Natural Spring Water (Stamford, CT) which also was used for all water trials.

Subjects

A total of 34 subjects (14 male, 20 female) between the ages of 21 and 63 participated in the experiments described herein. Subjects were recruited by word of mouth from the Philadelphia area. All subjects signed an informed consent, which, along with the protocols covering all experiments, were reviewed and approved by an independent, commercial institutional review board (Advarra, Seattle, WA). As part of the informed consent, subjects were told that they

would be participating in a taste test that proceeds as a game in which their success depends on their ability to detect and discriminate among different taste stimuli. They furthermore were informed of the taste stimuli they would encounter during the training and test sessions, as well as the earnings scheme and the minimum and maximum of possible earnings (see below).

On their first visit to the test site, subjects created a password-protected user profile that was entered into the database. To create the profile, subjects voluntarily entered information about personal traits selected from drop-down menus. The traits addressed in the profile were age, sex, height, weight, ethnic identity, zip code, and current medications. Completed profiles were automatically entered into the database; henceforward subjects logged-in to their profiles to begin a training or test session, and all data from the sessions automatically populated the database through each subject profile. Thus, all data points collected by the TāStation system were associated with the specific subject who generated the data, and also with all of the traits the subject entered into his/her profile.

Subjects were in visual and auditory contact with each other during sessions and no precautions were taken to prevent them from interacting with each other. Subjects were offered two cups, one with water and the other empty (for expectorating). Rinsing the mouth was not required at any time during a session, but instead was an option available to the subject as desired.

Prior to seating a subject, the TāStation system was readied for a session. The water-filled wash tube and 96-well plate containing the taste stimulus solutions appropriate to the particular training or test session were placed into the tray of the x-y motion table. A sterile filter pipette tip was attached to the end of the electronic pipette, and the pipette (pre-programmed to withdraw 200 μ l) was clipped into place on the z-axis gantry.

Subjects were instructed on how to handle and operate the system during a session—i.e., how to remove the pipette, how to dispense the contents of the pipette onto the tongue, replace the

pipette into the clasp of the z-axis gantry, and how to touch the TSD to record a response.

Subjects learned the rest of the procedure through experience with the gamified interactive algorithms.

Subjects were compensated with a base payment of \$25.00 for each training and test session plus earnings from the game in the sessions. The maximum amount of money that could be earned in a single training or test session was \$19.20, which resulted from correctly matching all the taste stimuli to the appropriate target on the screen. The \$25.00 per test base payment was sent the subject in the mail as a check from Opertech Bio at the end of the month(s) in which they participated in one or more sessions. Earnings from training or test sessions were paid to the subject by direct deposit into a PayPal account immediately upon completion of each session.

General Training Procedure

Immediately after the subject logged in, and the system initialized (which entailed automated checks on x-y table motion and communication with the electronic pipette), the x-y motion table moved the plate in a randomized pattern to align a single well of the 96-well plate with the pipette tip. Actuation of the z-axis gantry moved the pipette tip downward through the hole in the cabinet cover and into the well to withdraw a 200 μ l volume of sample from the well. The pipette then was raised to a position ready for the subject's grasp. A prompt "Remove Pipette" appeared on the TSD indicating to the subject that the trial had begun. The subject then removed the pipette from the clasp on the z-axis gantry and self-administered the sample to the tongue.

Training cues were mapped in the program to targets within the Cartesian plane on the TSD, defining the response field. The taste cue applied to the tongue indicated the target on the TSD the subject would have to touch in order to earn a virtual poker chip. The response field was

displayed on the TSD as a square with markings on the central x-y axes as depicted in Figure 2. The markings served only as a visual reference guide, roughly conveying dimensions of the grid. Either positive reinforcement or penalty occurred immediately upon registering a touch in the response field ending the trial. Touching the target correctly matched to its training cue taste stimulus resulted in positive reinforcement (the immediate appearance of the poker chip on the TSD, coincident with the sound of a cash register in operation represented by the onomatopoeia “cha-ching”), with the maximum, intermediate and low poker chip values of \$0.20 (bullseye), \$0.10 (middle ring), and \$0.05 (outer ring), respectively. Touches made outside the target, or on the wrong target, resulted in an immediate subtraction of \$0.10 from the cumulative score, coincident with an unpleasant buzzing sound and a timeout defined by a darkened screen and 15-second pause before the start of the next trial (Supplemental Video S2; if the quantity of errant responses within a session resulted in a negative value for the cumulative score by the end, the earnings were set to \$0.00—there was no deduction from the \$25.00 base payment). The x-y motion table then moved the wash tube into place beneath the pipette tip, which was lowered into the Eppendorf tube. The wash cycle consisted of one withdrawal and dispense action of 300 μ l of the water in the tube. Following the cessation of the wash cycle, the x-y motion table moved the plate to the next randomly selected well to begin the next trial (Supplemental Video S1). The actuation of the sample delivery device during a trial took approximately 15 seconds to complete.

The entire sequence and order of events in a trial thus consisted of 1) wash of the pipette tip in the wash tube, 2) movement of the x-y motion table according to a randomized pattern to align a single well beneath the pipette tip, 3) withdrawal of the sample (200 μ l) by the pipette from the well, 3) removal of the pipette from the z-axis gantry by the subject and self-administration of the sample to the tongue, 4) replacement of the pipette to the z-axis gantry, 5) recording the subject’s touch-response on the TSD, 6) consequence to the response.

No instruction or advice was given to subjects on where to apply the solutions on the tongue, and in the current studies, subjects were not required to spit out the sample after tasting and no stipulation was made on rinsing the mouth between trials—rinsing was entirely optional. There was no specified intertrial interval; subjects were allowed to take as much time as desired to make a touch response after tasting the sample and replacing the pipette. Regardless of the unlimited time available to make a response, subjects tended to respond within 10 to 15 seconds. Subjects were allowed to leave the testing suite to use the restroom in between trials if requested.

Subjects participated in as many training sessions as was necessary to achieve at least 90% taste target-appropriate responding, where target-appropriate responses were defined as touch responses placed anywhere within the target designated for the training cue presented on that trial. Subjects were considered ready for advancing to test sessions (where they would be presented novel stimuli—the test articles) when the 90% criterion was recorded for at least two consecutive training sessions.

General Test Procedure

For all experiments except Experiment 3, the discriminative stimulus cues from the training sessions also were included as control standards in the test session. On trials of the control standards, the contingencies of reinforcement and penalty remained in effect as described for training sessions. On trials of all test articles touch responses placed anywhere in the response field were reinforced by a maximum-value poker chip and the cash register sound. Each subject participated in two tests for Experiments 1 and 2A-2C, and 3-5 tests for Experiments 3 and 4.

Plate and Response Field Configurations for Experiments 1 and 2A-2C.

Training Plates. Solutions of sucrose (100 mM), NaCl (100 mM), citric acid (10 mM), and quinine (0.5 mM) each were dispensed in volumes of 290 μ l into 18 wells of a 96-well plate; the

remaining 24 wells were reserved for water (Supplemental Figure S1). These served as discriminative training cues in Experiments 1 and 2A. Training plates for Experiments 2B and 2C were identical except that the sucrose concentrations were 300 mM and 30 mM, respectively. The training cues were mapped in the program to targets within the Cartesian plane of the response field (detailed below).

Test Plate for Experiment 1. Water and solutions of sucrose (100 mM), NaCl (100 mM), citric acid (10 mM), and quinine (0.5 mM) served as control standards in the test plate. Water was dispensed in 8 wells of the 96-well plate, and the four other control standards each were dispensed in 6 wells. The remaining 64 wells were reserved for test articles—ranges of 8 concentrations each of sucrose, NaCl, citric acid and quinine. The concentration ranges were achieved by serial two-fold dilution from a top concentration that was anticipated to be maximally effective for each of the test articles. The top concentrations of the test article ranges were 500 mM for sucrose, 300 mM for NaCl, 30 mM for citric acid, and 1 mM for quinine. Each concentration of the respective ranges was dispensed in two wells of the 96-well plate (Supplemental Figure S2). All subjects were tested twice using this plate design, each test occurring on separate days.

Test Plate for Experiment 2A-2C. Control standards and their placement in the 96-well test plate for Experiment 2A were the same as described for the test plate of Experiment 1. The remaining 64 wells were reserved for test articles—ranges of 8 concentrations each of REB A, sucralose, ACE K, and sucrose. The concentration ranges were achieved by serial two-fold dilution from top concentrations that were anticipated to be maximally efficacious for each of the test articles. The top concentrations of the test article concentration ranges were 1 mM for REB A, 1 mM for sucralose, 3 mM for ACE K, and 500 mM for sucrose. Each concentration of the respective ranges was dispensed in two wells of the 96-well plate (Supplemental Figure S3.) The 96-well test plates for Experiments 2B and 2C were the same as for 2A except for replacement of the

100 mM sucrose control standard with 300 mM and 30 mM sucrose, respectively. All subjects were tested twice using these plate designs, each test occurring on separate days.

Response Field for Experiments 1 and 2A-2C. Four of the training cue targets each were placed in one of the four quadrants of the Cartesian plane of the response field; sucrose (100 mM), NaCl (100 mM), quinine HCl (0.5 mM), and citric acid (10 mM) were assigned to quadrants I, II, III, and IV, respectively. Water as a training cue was mapped to the origin of the Cartesian plane. The central x,y coordinates for each of the targets were specified in the program as a proportion of the distance across the abscissa and ordinates of the plane. Thus, the central coordinate pair for the target in quadrant I was 0.75, 0.25, that of quadrant II was 0.25, 0.25, in quadrant III, 0.25, 0.75, and in quadrant IV, 0.75, 0.75. The target placed at the origin of the grid was centered on 0.5, 0.5. The actual outer diameter of the target was approximately 2 cm, the diameter of the intermediate ring of the target was 1.5 cm, and the diameter of the bullseye surrounding the central coordinate pair was 1.0 cm. The targets were not displayed on the TSD. Subjects were required to discover their location through trial and error during the training sessions. The response field configuration was the same for both training and test sessions.

Procedure for Experiment 3

Experiment 3 was designed for estimating the lowest detectable concentrations of sucrose for each subject using a Method of Constant Stimuli (MCS) design. Therefore, there was no discrimination training session for this experiment.

Fourteen subjects (9 males and 5 females), 3 of whom were included in at least one of the previous taste discrimination experiments, participated in the MCS experiment. The locations of two targets, a sucrose-associated “sweet” target on the abscissa of the right side of the response field and the water-associated “not sweet” target on the left side were revealed to the subjects before the start of a test. The subjects were instructed to touch the “sweet” target if,

after pipetting a sample onto the tongue, they detected a “sweet” stimulus, and to touch the left-side target if the sample was not sweet. The stimuli were five different concentrations of sucrose (5, 10, 15, 20 and 25 mM) and water, presented to subjects in a randomized order. Each of 13 subjects was tested twice, and one subject tested once, for this experiment.

Test Plate. Sucrose solutions in concentrations of 25, 20, 15, 10 and 5 mM each were dispensed in volumes of 290 μ l into 12 wells of a 96-well plate; the remaining 36 wells were reserved for water (Supplemental Figure S4.).

Response Field. Two targets for the taste stimuli were programmed to occur on the central abscissa of the response field. The “sweet” target, assigned to all five of the sucrose solutions was centered at coordinates 0.75, 0.5. The “water” target, designated for water trials, was located at coordinates 0.25, 0.5.

Procedure for Experiment 4

Subjects for Experiment 4 previously had participated in Experiment 3. No discrimination training was given for Experiment 4. The testing procedure was essentially the same as for Experiment 3, except that a range of sucrose concentrations were presented as test articles and therefore all responses on those trials were reinforced.

Test Plate. Water and 200 mM sucrose served as the only control standards in the test plate. Each control standard was dispensed in 24 wells of the 96-well plate. The remaining 48 wells were reserved for test articles—a range of 8 concentrations of sucrose achieved by serial two-fold dilution from a top concentration of 500 mM (Supplemental Figure S5).

Response Field. The response field for Experiment 4 was the same as that described for Experiment 3. As with Experiment 3, targets were not displayed, and subjects were informed in advance of the target locations.

Data Analyses

The datum for all training and test sessions was defined as the number of touch responses made within the boundaries of the target of interest. Data were averaged across all trials within a test for each subject and also across test sessions and across subjects when required by the analysis.

For concentration-response analyses (Experiments 1, 2A-2C, and 4) curve fitting to datasets was achieved by nonlinear regression (GraphPad Prism, San Diego, CA) and EC50 values, Hill coefficients, and 95% confidence intervals (95%CI) were derived from the curve fit. The curve fitting model used for the regressions was a four-parameter variable slope model following the equation

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC50} - X) * \text{Hill Slope})})$$

where *log* refers to common log, here, throughout the text, and in all figures and tables.

Statistical determination of differences between pairs of concentration-response functions was achieved by an extra sum-of-squares F test, with the log EC50 selected as the parameter used as the basis for the comparisons (GraphPad Prism). Precision in the calculation of mean values for points used to fit curves in all figures with concentration-response functions is represented by 95%CI. The box and whisker plot of Figure 7 shows the interquartile range (from 25th to 75th percentile), median, and range as indications of variability.

Reproducibility of potency derived from concentration-response analysis was validated by the Replicate-Determination Study, following recommendations for assay development and validation detailed in the NIH Assay Guidance Manual (Haas et al., 2004). The pertinent statistics used in the analyses were the *Minimum Significant Ratio (MSR)*, the smallest potency ratio between two compounds that is statistically significant, and the *Limits of Agreement (LSA)*, an estimate of the repeatability of two measures.

MSR is determined by the equation

$$MSR = 10^{2s_d},$$

where s_d = the standard deviation of the paired differences in log potency across two concentration-response tests.

LsA was determined by the equation

$$LSA = 10^{\bar{d} \pm 2s_d},$$

where $10^{\bar{d}}$ = the geometric average fold-difference in potency across two concentration-response tests.

Values for discriminability, d' , were calculated for Experiments 3 and 4 according to the following equation:

$$d' = z(H) - z(F)$$

where $z(H)$ = the “hit rate, or true positive rate” (the proportion of responses made on the sucrose target on trials of sucrose) transformed to units of standard deviation (z score) by the inverse of the normal distribution function, and

$z(F)$ = the “false alarm rate, or false positive rate” (proportion of responses made on the sucrose target on water trials) transformed to a z score.

Differences between means in Experiment 3 were statistically evaluated by ordinary one-way ANOVA and Tukey’s multiple comparisons post hoc test.

RESULTS

Experiment 1

A chief aim of the first experiment was to determine whether robust taste discrimination could be achieved using 200 μ l sample volumes in a rapid throughput method. An initial indication of the

success of the method can be gained from inspection of the large body of data generated from hundreds of training sessions that have been conducted as of this writing. Mining the database for the two training sessions prior to any test for each of 34 subjects who had participated in at least two tests (including tests that were not part of the current study but that used the same training procedure) yielded a dataset from 226 sessions consisting of 21,696 data points. The proportions of correct responses on trials of each tastant used in the training procedure were 0.95, 0.96, 0.97, 0.98, and 0.98 for citric acid, NaCl, quinine, sucrose and water, respectively. Comparisons of error rates between trial 1 and trial 96 (Figure 3) showed that discrimination performance did not decline across the 96 trials of the training session. On the contrary, more errors were made on trial 1 than on trial 96 for all taste stimuli, suggesting that discrimination performance improved as the subjects progressed through the 96 trials; this was confirmed by examining the error rate, regardless of stimulus, across all 96 trials (Supplemental Figure S6.)

After establishing a rapid throughput procedure for discrimination among water and single concentrations of 4 different tastants, we tested a group of 8 subjects, each twice, for their ability to discriminate each tastant across a range of concentrations within the same test. All the tastants served both as control standards (requiring a correct response on those trials for positive reinforcement) and test articles (all responses on those trials were reinforced).

Concentration-response functions for discrimination were established for sucrose, NaCl, citric acid, and quinine demonstrating potencies ranging three orders of magnitude across the different tastants (Figure 4). Averaging data across all 16 tests (8 subjects, each tested twice) and curve-fitting with non-linear regression yielded EC50 values for sucrose, NaCl, citric acid, and quinine of 42 mM, 27 mM, 2 mM, and 64 μ M, respectively (Table 1).

The Hill coefficients (Table 1) derived from the non-linear regressions suggested a cooperative mechanism to the process of taste discrimination, rapidly accelerating as concentration

increased. After the acceleration, taste discrimination concentration-response functions for all four tastants saturated, as would be expected of a receptor-mediated process.

The relatively narrow 95%CI around the EC50 values (Table 1) suggested robustness to the assay which generated the functions. Since each of the 8 subjects conducted the test twice, we regarded the first and second sets of 8 tests as separate runs of the assay for the purpose of statistically evaluating re-test validity of the assay. To this end, the replicate-experiment analysis (Eastwood et al., 2006), adapted for *in vivo* assays with 3 to 5 compounds (Haas et al., 2004) was conducted for the two runs to determine the MSR and LsA. The acceptance criteria for reproducibility of test results are an MSR less than or equal to a value of 3, and an LsA ranging between values of 0.33 and 3.0. Values for MSR and LsA calculated from the ratios of EC50s for runs 1 and 2 were 1.75 and 0.66, respectively, and therefore the assay achieved a standard of validity for generating reliable *in vivo* concentration response functions.

Experiment 2

Experiment 2A. Eight subjects, 3 of whom had participated in Experiment 1, were trained using the same procedure as described for Experiment 1 and tested for their ability to discriminate among multiple TAS1R2/R3 agonists within the same test session. Concentration-response functions for taste discrimination of four TAS1R2/R3 agonists, sucrose, REB A, sucralose, and ACE K, were established (Figure 5), demonstrating a broad range of potencies (Table 2). A replicate-experiment analysis comparing the first and second tests yielded an MSR of 1.67 and LsA of 0.68, meeting the standard measure for assay repeatability.

Experiment 2B. Next, 8 subjects, 4 of whom had participated in Experiment 2A, were trained as previously, except that 300 mM sucrose replaced 100 mM sucrose as the discriminative training cue representing sweet taste quality. All subjects met the 90% accuracy criterion and therefore were test-ready within two training sessions. Each of the 8 subjects was tested twice using the

same experimental design and test-plate, again with the exception that 300 mM sucrose replaced 100 mM as a control standard. Data averaged across all 16 tests were used to generate concentration-response functions for sucrose-target responding as shown in Figure 6A. The resulting functions and potencies (Table 2) were essentially equivalent to those generated in Experiment 2A where 100 mM sucrose was the training cue and control standard. The data from the first 8 tests and those of the second 8 tests of this experiment also were entered into the replicate-experiment analysis used in Experiment 1 to test the run-to-run repeatability of assay results. Calculated values for MSR and LsA were 1.09 and 0.87, respectively, and thus the assay met the standard for acceptable repeatability.

Experiment 2C. Next, 8 subjects, all of whom had been in either Experiment 2A, 2B, or both, were trained as before but with 30 mM sucrose as the discriminative training cue representing sweet taste quality. Three subjects reached the test criterion of 90% or greater target-appropriate responding for 2 consecutive training sessions within 2 days, whereas the remaining 5 subjects required additional sessions. The median number of training sessions needed to reach test criterion was 3, but ranged as high as 37 with one subject, suggesting the 30 mM sucrose cue was more difficult to discriminate than either 100 or 300 mM sucrose as in the previous experiments. Upon achieving test-readiness, subjects were tested in a concentration-response analysis of the four TAS1R2/R3 agonists with 30 mM sucrose as the control standard. The resulting concentration-response functions resembled those of Experiments 2A and 2B, with the same order of potencies. However, the functions tended to be relatively left-shifted, with statistically detected differences (extra sum of-squares F test, $p < 0.0001$) between EC50s from Experiments 2B and 2C for ACE K, REB A, and sucrose (Table 2).

In sucrose control standard trials in test sessions of Experiment 2A (100 mM) and 2B (300 mM), proportions of sucrose-appropriate responses were 0.90 and 0.99, respectively (see Figure 6A

and 6B). In contrast, target-appropriate responding on sucrose control standard trials (30 mM) in test sessions of Experiment 2C fell to 0.49. It is important to emphasize that all subjects had reached the test-ready criterion of correctly associating the 30 mM sucrose cue with its designated target on over 90% of trials in training sessions just prior to testing.

An MSR value of 1.36 and LsA of 0.79 were calculated from the ratios of EC50s for runs 1 and 2 of Experiment 2C, achieving the standard of validity for *in vivo* concentration response functions.

Experiment 3

The analyses carried out in Experiments 1, 2A, 2B, and 2C consistently yielded EC50 values for taste-discrimination of sucrose that ranged around 30 mM, yet subjects were able to achieve 90% target-appropriate responding on trials of 30 mM sucrose when it was used as the discriminative cue in training sessions (Experiment 2C). To gain further understanding of the discriminability of sucrose at the lower portion of the concentration-response function a Signal Detection Theory (SDT) method of continuous stimuli (MCS) was used to focus on and obtain d' values for concentrations of sucrose below 30 mM.

The proportions of responses occurring on the sucrose target on trials of water and the five concentrations of sucrose recorded across all 27 tests are plotted in Figure 7. Subjects readily identified trials of 25, 20, and 15 mM as sweet, with proportions of correct “sweet” responses—“true positives”—close to 1.0 for all three concentrations. Significantly fewer sweet responses occurred on trials of 10 mM and 5 mM sucrose (one-way ANOVA, 10 mM vs. 15 mM, $p < 0.027$; 5 mM vs. 15 mM, $p < 0.0001$, $df = 156$). Proportions of target-appropriate responses on trials of 5 mM sucrose ranged from 0.83 to 0.33 with a median value of 0.50; “sweet” responses also were recorded on trials of water (i.e., “false positives”), with proportions ranging from 0.55 to 0.03, and a median value of 0.31 (Figure 7). The ranges of proportions for “sweet” responses on 5

mM sucrose and water trials overlapped but were significantly different (one-way ANOVA, $p < 0.001$, $df = 156$). Thus, 5 mM sucrose appeared to be discriminable from a background of water for most of the subjects, but probably near an empirical threshold for detection. An SDT analysis of discriminability, which takes response bias into account, as was observed in the false positive rate on water trials in this experiment, was applied to the group data; the resulting d' value for 5 mM sucrose was 0.67 (Table 3).

In contrast, 25 mM was discriminated from water with nearly perfect accuracy (4 errors out of 324 trials across all subjects), with a calculated d' value of 2.83 (Table 3). Thus, under conditions of a binary choice between “sweet” and “not sweet” stimuli, where correct choices are reinforced and incorrect choices penalized, concentrations of sucrose that are just below EC50 values in the concentration-response functions of Experiments 1 and 2A–2C were readily detected.

The results from the MCS appeared to be at odds with those obtained from the concentration-response analyses conducted in Experiments 1 and 2A–2C. To more closely examine the conditions that determine the concentration-dependence of sucrose discriminability, an additional concentration-response analysis was performed that combined elements of both the MCS of Experiment 3 and the concentration-response design of the previous experiments.

Experiment 4

Eight subjects (6 males and 2 females) of the MCS experiment (Experiment 3) participated in a concentration-response test in which 200 mM sucrose and water served as control standards and 8 different concentrations of sucrose served as test articles. Instructions given to the subjects for this test were identical to those for the MCS experiment—subjects were to make a binary choice between the “sweet” and “not-sweet” targets after pipetting a sample onto the tongue. Each subject completed 2 tests for this analysis.

Results from the concentration-response analysis performed on data collected across all 16 tests of this experiment are presented graphically in Figure 8. The proportion of “sweet-target” responses on control trials of 200 mM sucrose and water were 1.00 and 0.05, respectively. The curve-fit of data from test article trials yielded a potency value for sucrose (33 mM; Figure 8) that was essentially equivalent to that obtained in Experiment 2C (31 mM; Table 2), and similar to those of Experiments 1, 2A and 2B. Therefore, reducing the task to a binary choice between two stimulus categories, water and “sweet,” had little, if any, substantive impact on the location of the EC50.

Threshold measurements often are taken as an indication of an individual’s taste sensitivity. To examine the relationship, if any, between an individual’s sensitivity to low concentrations of sucrose (i.e., near an empirical threshold) and an individual’s EC50, two additional MCS and binary concentration-response tests were conducted using the same 8 subjects to obtain reliable individual measures for each subject. Individual subject values for d' and EC50 were calculated from the proportions of responses occurring on the “sweet” target cumulative across the four tests. The results suggest little correspondence between the 5 mM d' and the EC50 (Table 4). For example, the 5 mM sucrose d' for subjects M1176 and M1194 were similar (0.60 and 0.64, respectively), but their EC50s (51 mM and 21 mM, respectively) were significantly different (extra sum of-squares F test, $p < 0.0001$.) Moreover, the subject with the highest d' of the group (subject M1013), and thus the most sensitive to sucrose taste by that measure, generated an EC50 of 37 mM, whereas a lower EC50 (28 mM) was obtained from the subject with the lowest d' (i.e., lowest sensitivity) of 0.26 (subject F1099).

Thus, the “sensitivity” of a subject to the taste of sucrose, as determined by d' at or near an empirical threshold concentration, is not reflected in the placement of the EC50, which is derived from the curve fit to multiple suprathreshold concentrations. The 5 mM d' of Experiment 3 appears to better correspond to the lowest concentrations of the concentration-response

function, 3.9 and 7.8 mM, where the curve is anchored and begins to accelerate above baseline (see Figure 8).

DISCUSSION

Enabled by the rapid throughput technology and procedure, taste discrimination concentration-response functions were generated for sucrose, citric acid, quinine, and NaCl, stimuli representative of the basic taste qualities “sweet,” “sour,” “bitter,” and “salty,” respectively, each known to signal through independent sensory cell types (reviewed in (Palmer, 2019; Roper and Chaudhari, 2017)). Concentration-response functions also were established for the TAS1R2/R3 receptor agonists ACE K, REB A, and sucralose. The resulting EC50s are comparable to those obtained from cell-based assays of recombinant TAS1R2/R3 (Li and Servant, 2008; Palmer, 2019). All functions were contained within a span of approximately two orders of magnitude, the range expected of a bimolecular interaction between an agonist and a single binding site quantitatively described by mass action law (Kenakin, 2018; Limbird, 2005). The Hill slope of the functions for all tastants was greater than unity, suggesting a cooperative mechanism occurring somewhere in the signaling process (Karnani and Jackson, 2018). Cooperativity as indicated in these data could result from agonist-receptor interactions (Gesztelyi et al., 2012), but could also reflect physiological processes that amplify sensory signals along the pathways of their propagation (Adair, 2001). Steep dose-response functions in drug discrimination experiments frequently are observed, potentially resulting from the schedules and contingencies of reinforcement of those procedures (Barrett et al., 1994; Mathis and Emmett-Oglesby, 1990).

Several non-nutritive sweeteners have been reported by humans to impart aversive off-tastes (Horne et al., 2002), and also have been shown to generalize to a quinine-like cue in a subset of individual Sprague Dawley rats in taste discrimination experiments (Loney et al., 2012). In Experiments 2A-2C of the current study, no responses on the quinine-trained “bitter” target were recorded on trials of non-nutritive sweeteners—all responses across subjects occurred either on

the sucrose-trained “sweet” target or the water target, in a concentration-dependent manner. These results should not be interpreted as an absence of, or failure to detect, additional stimulus properties among the non-nutritive sweeteners that are not sucrose-like (“pure sweet”) and potentially aversive. Subjects were forced to choose the one target that best represented the stimulus properties of the tastant. Aversive off-tastes of non-nutritive sweeteners are revealed using our procedure if a target associated with mixtures of sucrose and quinine is incorporated into the experimental design (unpublished results).

Single concentrations of tastant were used in our studies as the training cue (training sessions) and control standard (test sessions). Given the significance of training dose for drug discrimination (Colpaert et al., 1980; Stolerman et al., 2011; Vanecek and Young, 1995), we considered the possibility that a different training concentration of tastant could yield different concentration-dependent patterns of stimulus generalization in human taste discrimination. Changes in the sucrose training concentration between 100 and 300 mM had little, if any, impact on the concentration-response functions of the TAS1R2/R3 agonists tested here (Table 2). However, statistically detectable leftward shifts in the potencies of sucrose, ACE K, and REB A were observed (a lower EC50 value was recorded for sucralose in Experiment 2C compared to 2B, but the shift did not meet the standard of significance.) This outcome might have been a behavioral consequence of contingencies of reinforcement in place for trials of 30 mM sucrose presented as a control standard during the tests. On those six trials (see plate design of Figure S3), only “correct” responses (“sweet” target) were reinforced, and “incorrect” responses (water target) were punished. Responses to test articles at stimulus-equivalent concentrations in the linear portion of the concentration-response functions therefore would have been influenced toward greater frequencies of occurrence on the “sweet” target.

Although 30 mM sucrose was more difficult to acquire than 100 and 300 mM as a training cue, all subjects of Experiment 2C eventually met test-ready criterion. But the high level of

discriminatory performance on 30 mM sucrose trials of training sessions did not carry over into the test sessions. Instead, responses on control standard trials of 30 mM fell to approximately 50% in the test sessions. In other words, responding to 30 mM sucrose defaulted to that of an EC50 when tested along with the full range of taste-active concentrations.

The relative decline in the rate of “sweet” target responses on control trials of 30 mM sucrose in test sessions could have been due to the contextual impact of higher concentrations of sucrose presented among the trials (Riskey et al., 1979; Schifferstein and Frijters, 1992). Unlike the training sessions of Experiments 2A-2C, all test sessions included 125, 250, and 500 mM sucrose presented as test articles. If the context of higher concentrations was a factor in determining the discriminability of lower concentrations, the effect was only apparent within the linear portion of the concentration-response function. Concentrations of sucrose between 125 and 500 mM were equivalent and saturating with respect to discriminability, an observation consistent with a receptor-occupancy explanation of the characteristics of the concentration-response function for taste discrimination. This explanation does not necessarily imply that high concentrations of tastant cannot be discriminated from each other in a direct comparison, but that taste-quality plays no further role in the discrimination. As tastant concentrations continue to rise beyond saturation so will the rates of stimulus onset, a potentially detectable discriminatory cue. Furthermore, as suggested by (McBride, 1983), changes in viscosity of sucrose solutions at high concentrations could provide a discriminable orosensory cue unrelated to taste.

A tenet of SDT portrays sensory signals as carriers of information, and the value that information has to a perceiving organism will contribute to the setting of a criterion demarcating a boundary between legitimate signal and background noise. The value of the signal will be a function of the consequences of responding (or failing to do so) to the sensory stimulus (Macmillan and Creelman, 2005). It is therefore reasonable to expect contingencies of reinforcement to impact measures of discriminability. Signal detection by a method of constant

stimulus was conducted in Experiment 3, in which all trials of a binary choice between the sweet and water targets were governed by control standard contingencies of reinforcement. The highest in the range of concentrations tested, 25 mM, was detected on nearly 100% of trials, and thus the contingencies of reinforcement and punishment might have enhanced discriminability of sucrose at the low end of the equivalent range in the concentration-response functions. Nevertheless, any detection-enhancing impact of the reinforcement contingencies was bounded by a lower limit in the vicinity of 5 mM. This lower limit is consistent with a response probability occurring at the lowest levels of receptor occupancy and further corresponds to the lowest portion of the concentration-response functions of all other experiments in the current study.

Detection thresholds often are taken as the primary indication of the physiological sensitivity of the taste system to a stimulus, but the relationship between taste thresholds and the potency of a tastant agonist has been unclear. A leftward shift of the tastant concentration-response function, resulting from receptor-dependent factors such as agonist affinity and intrinsic efficacy (Black et al., 1985), would be one plausible mechanism accounting for relatively low thresholds of detection. The design of Experiment 4 tripled the number of replicates, thereby improving the resolution of the assay such that reliable potency determinations for sucrose were established for each individual subject. We continued to test the 8 subjects of Experiment 4 to generate an additional 2 concentration-response functions and 2 more MCS experiments. Collapsing data across all 4 of each test, cumulative d' and EC50 values were calculated for each individual subject. The cumulative values thus obtained should be less impacted by inherent day-to-day variability than would be the case for single determinations, often the practice of traditional threshold testing. The data presented in Table 4 suggest little in the way of a clear correspondence between a subject's ability to detect sucrose at or near threshold and the potency of sucrose in taste discrimination. Therefore, sensitivity to the lowest detectable

concentrations does not functionally distribute to suprathreshold concentrations in taste discrimination. However, only a portion of the variability in threshold values across individuals should be related to receptor-dependent mechanisms, as many of the subject-dependent variables associated with taste thresholds in the literature, such as sex (Joseph et al., 2016), age (Yoshinaka et al., 2016), ethnicity (Williams et al., 2016), and endowment of lingual tissue (Zhang et al., 2008) bear no obvious relationship to receptor function. A convergence of the two experimental endpoints of taste threshold and EC50 would suggest a receptor-dependent mechanism, perhaps confirmed by genetic analysis.

Generally, there are two sources of error in any measurement: 1) stochastic variation inherent to the system being studied, and 2) the resolving power of the tool which is used to measure the system. For human taste testing, increasing the number of subjects can reduce the impact of both general sources of variation to achieve statistical resolution. In our experiments, we used cohorts comprised of eight subjects that were not pre-screened for any particular trait; other than trying to balance the numbers of males and females for the cohorts, selection of subjects predominantly was based on their availability. As we have noted here, many subject-dependent variables have been claimed to influence taste, pointing to wide ranging sources of intersubject variability. Nevertheless, our results were lawful and robust, plausibly resulting from the greater density of data per subject. The 95%CI surrounding the potency determinations for sucrose were relatively narrow, suggesting that the concentration-response functions obtained in these experiments were not greatly impacted by fluctuating subject-dependent variables among the cohorts.

A pharmacological approach to taste, enabled by robust concentration-response analysis, can further be applied to settling the science around other open questions in taste research, such as confirming and quantifying the effects of putative taste inhibitors or enhancers. For example, amiloride, an inhibitor of the epithelial sodium channel (ENaC), has been useful in associating

ENaC function to detection of sodium taste in mice (Chandrashekar et al., 2010; Shigemura et al., 2008). However, its use in human testing has produced equivocal results, adding to uncertainty about the role of ENaC in human salt taste (reviewed in (Bigiani, 2020)). Using the methodology reported here, shifts in the concentration-response function for NaCl taste discrimination should be evident if amiloride is pharmacologically active on the mechanism underlying sodium taste in humans. Similarly, a variety of compounds have been reported to antagonize TAS2R receptors *in vitro*, but as previously pointed out (Palmer, 2019) few have been demonstrated to block bitter taste *in vivo*, and among those, the reported effects have been modest. TAS2R antagonists should measurably impact the concentration-response functions for bitter taste discrimination, an effect that will be most pronounced when paired with agonists that show any receptor selectivity. Such an approach could further help to relate tastant receptor to *in vivo* taste responses, associations that have been unambiguously established for only a fraction of the 25 human, or 30 murine, TAS2R subtypes (Palmer, 2007; 2019).

In summary, robust concentration-response functions for taste discrimination, consistent with the hyperbolic functions predicted by receptor occupancy theory, have been generated using human subjects. Reliable tastant potencies derived from the functions permit a pharmacological characterization of tastant agonists and should prove useful for quantifying ligand-receptor interactions of other taste-regulating compounds across a spectrum of efficacies.

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AUTHORSHIP CONTRIBUTIONS

Responsible for research design: Palmer

Conducted experiments: Stewart, Talley, Palmer

Contributed new analytic tools: Palmer

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FIGURE LEGENDS

Figure 1. Apparatus. (A) Sample delivery device (left, with electronic pipette mounted in z-axis gantry) and laptop on table as would be positioned before a subject. **(B)** Sample delivery device viewed from front with lid to x-y motion table cabinet removed. A 96-well plate is visible, nested in the tray attached to the x-y motion table. An Eppendorf tube filled with water for washing the tip is inserted into a holder at the front of the tray; the pipette is shown withdrawing a sample from a well at the start of a trial.

Figure 2. Laptop Touch-sensitive Display (TSD) as it appears during a training or test session. Response field appears on the left side of the display; the actual abscissa and ordinate of an underlying Cartesian plane is projected. The hatch marks on the abscissa and ordinate are for visual guidance only and do not correspond to numeric values. An 8 x 12 matrix, which tracks trial-by-trial progress through the game-like session, appears to the right of the response field. The consequence (indicated by a red "X" if negative, and a small poker chip if positive) on each trial appears in the next open cell immediately after the trial's end. In this example, the subject has correctly associated the taste stimulus with its designated target by touching a location in the center of the response field (coordinates of 0.5,

0.5), resulting in the immediate appearance of a large poker chip just below the 8 x 12 matrix. Counters appear below the left and right corners of the 8 x 12 matrix; left counter tracks remaining trials in the progression and right counter displays cumulative points earned (each point worth \$0.01). The bar above the 8 x 12 matrix displays messages to prompt the subject to take a particular action needed in the sequence of events for a trial.

Figure 3. Comparison of Percentage of Errors on Trial 1 and Trial 96 of Training Sessions. Data are cumulative across 226 training sessions of 34 subjects who had been trained prior to a test using Training Protocol 2. Numbers above each bar indicate the number of errors divided by the number of trials on which the tastant was presented on trial 1 or trial 96. The number of sessions for each individual subject comprising this dataset ranged from 3 to 22. **A)** 10 mM citric acid; **B)** 100 mM NaCl; **C)** 100 mM Sucrose; **D)** 0.5 mM quinine.

Figure 4. Concentration-response functions for taste discrimination of tastant agonists representing stimuli of basic tastes. Test articles: ranges of 8 concentrations were created for quinine (QUI), citric acid (CIT), sucrose (SUC), and NaCl by serial 2-fold dilution. Controls standards: QUI (CS) = 0.5 mM quinine, CIT (CS) = 10 mM citric acid, NaCl (CS) = 100 mM NaCl, SUC (CS) = 100 mM sucrose. Data are plotted as the proportion, **p**, of responses that occurred on the target designated for each of the tastants. Each data point in the curves was calculated as the average across 32 replicates (2 replicates per test x 2 tests x 8 subjects). Curves were fit to the data points by non-linear regression. Points for control standards (CS) are plotted on the left of the graph, and were calculated as the average across 96 (6 replicates per test x 2 tests x 8 subjects) for QUI (CS), CIT (CS), NaCl (CS), and 128 replicates (8 replicates per test x 2 tests x 8 subjects) for water. The data point for water is the

proportion of responses occurring on any target other than water (false positives). Error bars are 95%CI.

Figure 5. Concentration-response functions for taste discrimination of TAS1R2/R3 agonists.

Test articles: ranges of 8 concentrations were created for sucrose (SUC), acesulfame potassium (ACE K), sucralose (SCRL), and rebaudioside A (REB A) by serial 2-fold dilution. Data are plotted as the proportion, p , of responses that occurred on the target designated for each of the tastants. Each data point in the curves was calculated as the average across 32 replicates (2 replicates per test x 2 tests x 8 subjects). Curves were fit to the data points by non-linear regression. Points for control standards (CS) are plotted on the left of the graph and were calculated as described in caption to Figure 4. The data point for water is the proportion of responses occurring on any target other than water (false positives). Error bars are 95%CI. Data points for control standards of quinine, citric acid and NaCl, each surpassing a proportion of 0.9 target-appropriate responses, are omitted from the figure for clarity.

Figure 6. Concentration-response functions for taste discrimination of TAS1R2/R3 agonists with 300 mM vs. 30 mM sucrose as the control standard for training and testing. Data are plotted as described in caption to Figure 5. **(A)** “Sweet” control standard 300 mM; **(B)** “Sweet” control standard 30 mM. Error bars are 95%CI. Data points for control standards of quinine, citric acid and NaCl, each surpassing a proportion of 0.9 target-appropriate responses, are omitted from the figure for clarity.

Figure 7. Detection of sucrose concentrations below EC50 by method of continuous stimuli (MCS). Data are plotted as proportions of responses made on the sucrose target on trials of each stimulus, cumulative from 27 tests (13 subjects tested twice; one subject tested once). Total number of trials that generated the dataset were 324 for each concentration of sucrose (12 per test), and 972 for

water (36 per test). “Correct” responses were reinforced, and errors were penalized. Boxes indicate interquartile range from 25th to 75th percentile; median is indicated by horizontal line within the box; whiskers indicate range.

Figure 8. Concentration-response functions for taste discrimination of sucrose in a binary “sweet” vs. “not sweet” task. Data are plotted as the proportion of responses that occurred on the target designated for sucrose (the “sweet” target). Error bars are 95%CI. Each concentration of each tastant used to generate the functions was presented in 6 replicates per test. Data points were averaged across 16 tests from 8 subjects, each tested twice (total of 96 replicates per data point.) Control standards of 200 mM sucrose and water each were presented on 24 trials per test. Data from control trials are plotted on the left; the data point for water is the proportion of responses occurring on the “sweet” target (false positives). Analysis by nonlinear regression of the data points yielded curve-fit parameters for EC50 of 33 mM (95%CI of 29-37 mM), Hill Slope of 2.64 (95%CI of 2.01-3.62), and an R² coefficient (goodness of fit) of 0.90.

TABLE 1. Curve fit parameters of concentration-response functions from Experiment 1.

EC50, Hill slope, and goodness of fit (R^2) values derived from nonlinear regression of the concentration-response data for discrimination of “basic taste” stimuli. Values in parentheses represent 95%CI.

| | <u>Sucrose</u> | <u>Quinine</u> | <u>Citric Acid</u> | <u>NaCl</u> |
|----------------------|--------------------|------------------------------|--------------------|--------------------|
| EC50 | 42 mM (37 - 48 mM) | 64 μ M (44 - 88 μ M) | 2 mM (1 - 3 mM) | 27 mM (19 - 36 mM) |
| Hill Slope | 2.40 (1.32 - 3.49) | 1.98 (1.03 - 2.93) | 2.75 (1.33 - 4.14) | 2.25 (1.10 - 3.40) |
| R² | 0.76 | 0.74 | 0.74 | 0.71 |

TABLE 2. Comparison of EC50s for TAS1R2/R3 agonists from experiments using different concentrations of sucrose as the training cue and control standard. Sucrose training cue and control standard for Experiment 2A = 100 mM; for 2B = 300 mM; for 2C = 30 mM. Values are given as EC50s derived from nonlinear regression of the concentration-response data for discrimination of REB A (rebaudioside A), SCRL (sucralose), ACE K (acesulfame potassium), and SUC (sucrose). Values in parentheses represent 95%CI.

| | <u>Experiment 2A</u> | <u>Experiment 2B</u> | <u>Experiment 2C</u> |
|--------------|---------------------------------|---------------------------------|---------------------------------|
| REB A | 56 μ M (44 - 73 μ M) | 60 μ M (53 - 69 μ M) | 30 μ M (26 - 34 μ M) |
| SCRL | 79 μ M (64 - 99 μ M) | 132 μ M (96 - 178 μ M) | 98 μ M (90 - 107 μ M) |
| ACE K | 310 μ M (251 - 380 μ M) | 381 μ M (329 - 441 μ M) | 153 μ M (125 - 187 μ M) |
| SUC | 40 mM (31 - 52 mM) | 51 mM (45 - 59 mM) | 31 mM (28 - 34 mM) |

TABLE 3. Discriminability of sucrose concentrations below EC50 obtained by Signal

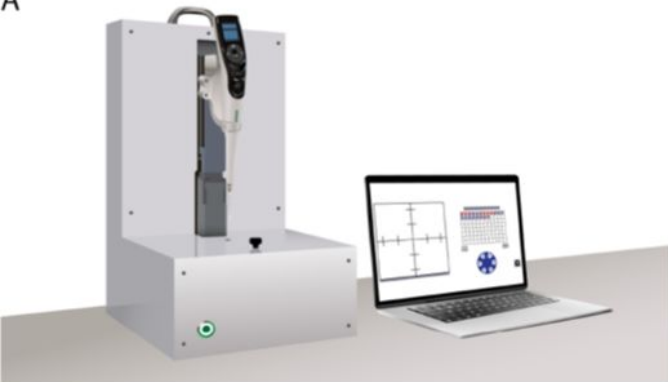
Detection Theory method of constant stimuli. Subjects were presented five concentrations of sucrose and water in a randomized order and recorded responses on either the “sweet” target or “water” target in the response field of the touch-sensitive display. All “correct” responses (touching the “sweet” target on trials of any concentration of sucrose or the “water” target on trials of water) were reinforced and “incorrect” responses were penalized (as described in Methods.) The value for discriminability, d' , which takes response bias into account (see Data Analysis), was calculated from the cumulative results of 27 tests (13 subjects tested twice; one subject tested once).

| <u>[Sucrose] (mM)</u> | <u>d'</u> |
|------------------------------|-------------------------------|
| 25 | 2.83 |
| 20 | 2.45 |
| 15 | 2.05 |
| 10 | 1.57 |
| 5 | 0.67 |

TABLE 4. Comparison of d' (5 mM) and EC50 values for sucrose measured in individual subjects. Results given were obtained from 8 subjects, each identified by their subject ID code. The d' at 5 mM was calculated from data cumulative across 4 MCS tests (see Methods for Experiment 3) for each subject; EC50 values and 95%CI, given in units of mM, were determined by averaging across results from 4 concentration-response tests (see Methods for Experiment 4) for each subject. A confidence interval was not returned by the regression analysis for subject M1200 due to the steepness of the curve fit to that individual's dataset.

| <u>Subject</u> | <u>d'</u> | <u>EC50 (95%CI)</u> |
|-----------------------|-------------------------------|----------------------------|
| M1013 | 1.66 | 37 (32-43) |
| M1198 | 0.42 | 54 (48-59) |
| M1176 | 0.60 | 51 (45-56) |
| M1194 | 0.64 | 21 (13-29) |
| M1200 | 0.42 | 30 (---) |
| M1018 | 0.53 | 31 (26-37) |
| F1005 | 1.51 | 19 (16-23) |
| F1099 | 0.26 | 28 (23-33) |

A



B

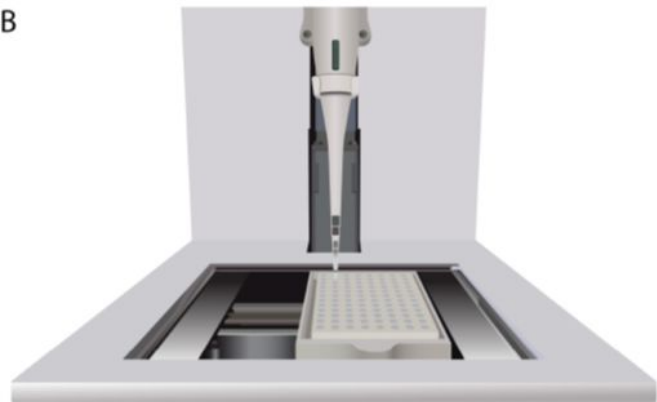


Figure 1

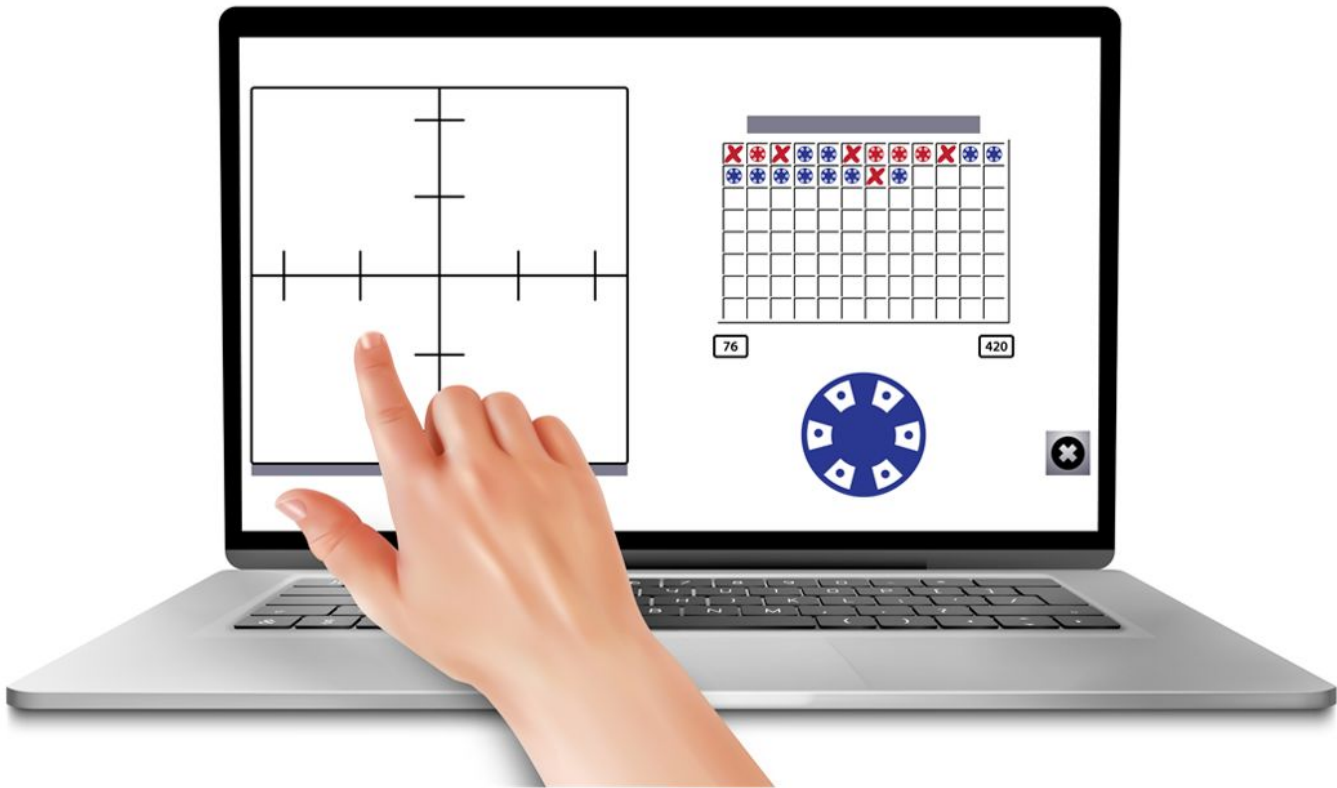


Figure 2

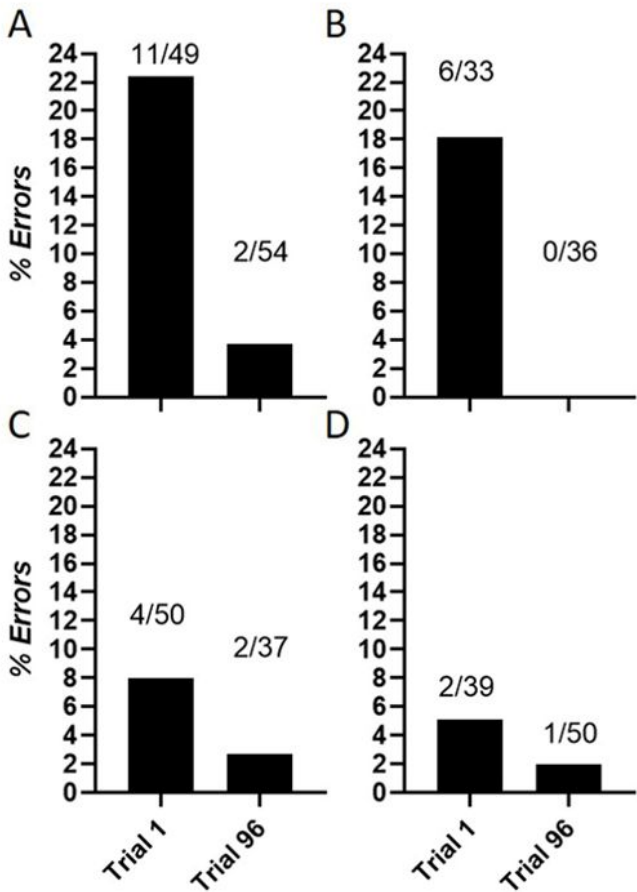


Figure 3

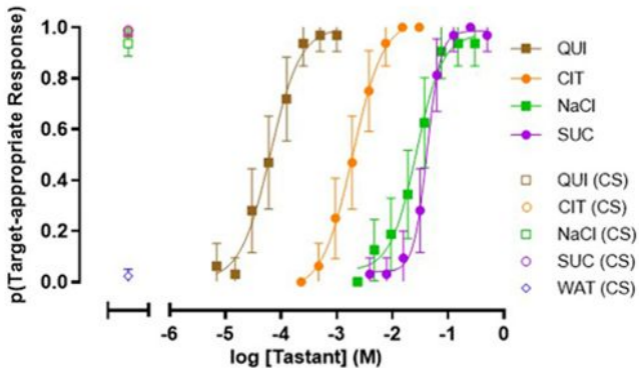


Figure 4

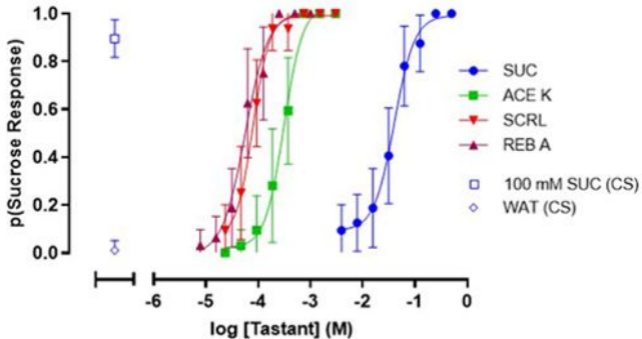


Figure 5

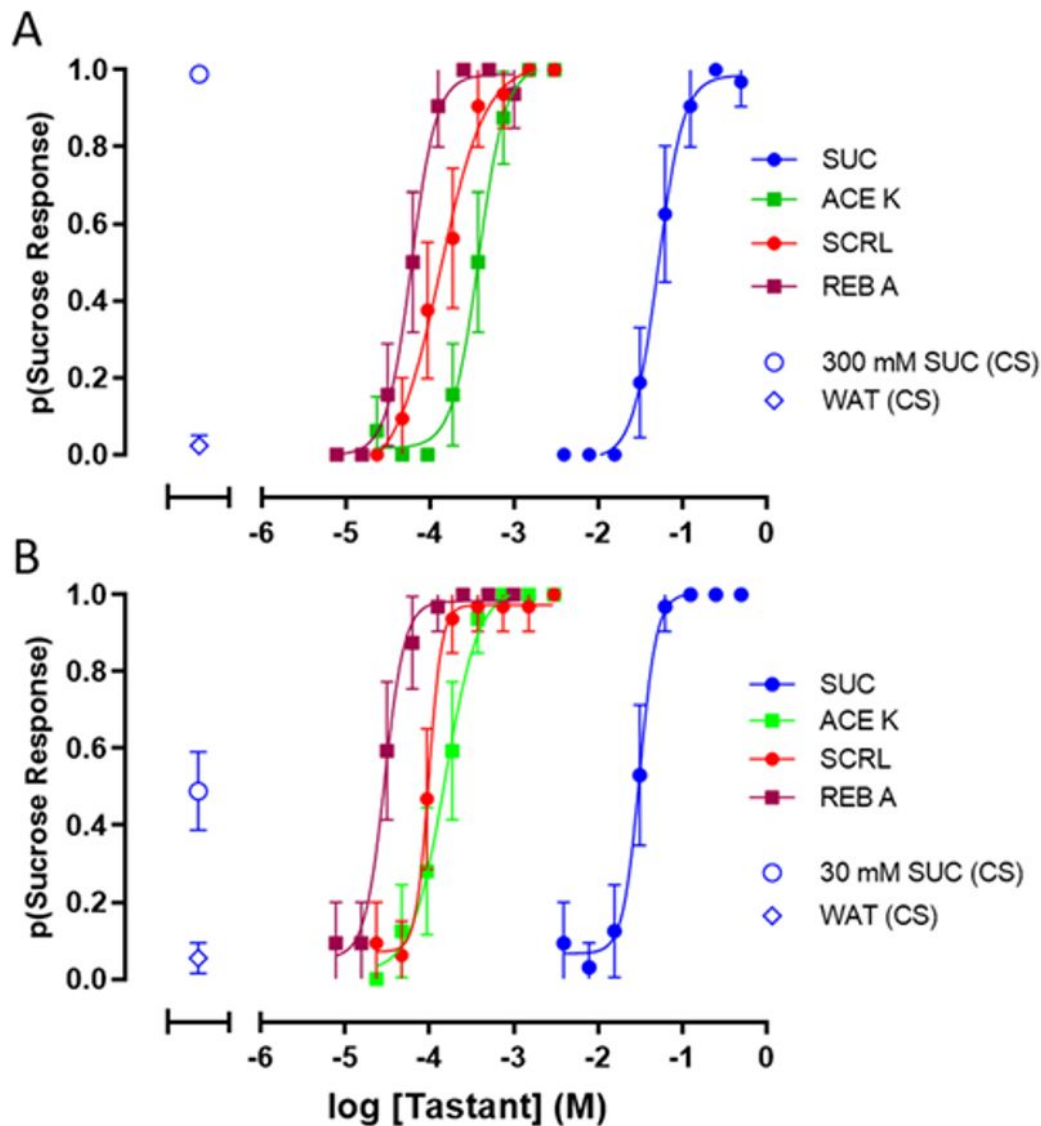


Figure 6

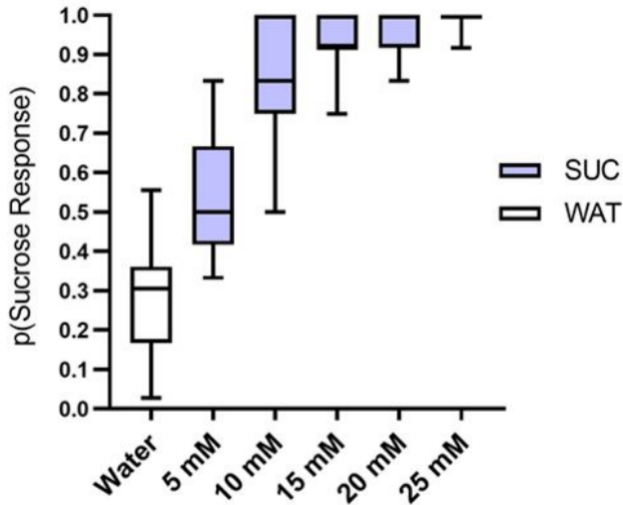


Figure 7

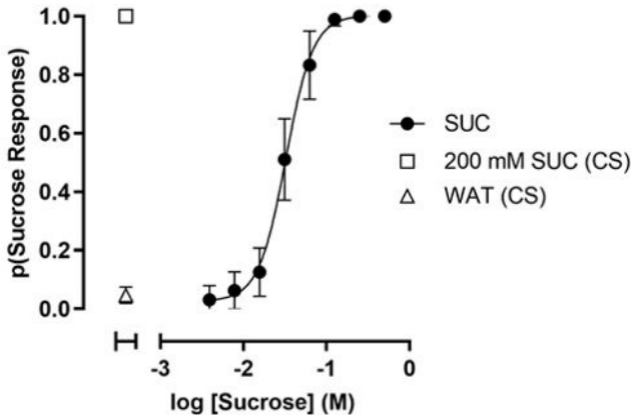


Figure 8